Review of Report, "Testing the Taxonomic Validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*)," by R. R. Ramey II, H-P. Liu & L. Carpenter

Review Submitted by Mary V. Ashley, Ph.D., Dept. of Biological Science, University of Illinois at Chicago

Question 1: Do the morphological, ecology, and mtDNA data presented in the report support the authors' conclusions on synomyzing Z. h. campestris and Z. h. preblei?

Although I am not an expert on morphological data analysis, I found that the authors' strongest case for synomyzing the two subspecies comes from their thorough morphological study. Discriminant function analysis based on 33 *Z. h. preblei* and 39 *Z. h. campestris* specimens had poor discriminating ability, correctly assigning only 48% of specimens. It should be noted that the authors applied a fairly strict criterion of p>0.95 posterior probability. However, it does not appear that the two subspecies are morphologically distinct. This is a fairly straightforward conclusion based on solid data set.

A cautionary note on morphology, however, comes from the work of Conner and Shenk (2003). They were able to develop a discriminant function based on repeated measures (single measurement sets were not sufficient) to distinguish between *Z. h. preblei* and another species, *Zapus princeps princeps*. They report a 70% error rate in species identification for specimens from southeastern Wyoming. The point is that *Zapus* species and subspecies are morphologically very similar.

With regards to ecological distinctiveness, the authors present no information either way. Although they use lack of evidence in the literature for ecological differences to argue for synomyzing, they present no references and it is difficult to judge how thoroughly they looked. Did they include the 'gray' literature and technical reports? At this point, lack of evidence represents lack of information, not evidence *for* synomyzing.

With regards to the mtDNA data, the results are interesting and can be interpreted differently depending on one's viewpoint. The author's position is that *Z. h. preblei* and *Z. h. campestris* are not reciprocally monophyletic for mtDNA haplotypes, and no unique mtDNA haplotypes were found in *Z. h. preblei*. This is the crux of their argument for synonmyzing the subspecies based on genetic data, and based on very strict criterion, it is a valid argument. However, inspection of the results in Figure 2 and Table 1 indicate that there are four *Z. h. preblei* haplotypes and these are all at fairly high frequencies within *preblei*. While these haplotypes are also found in *Z. h. campestris*, the shared haplotypes are at very low frequency in *Z. h. campestris*. Clearly, based on haplotype *frequencies*, the two 'subspecies' are genetically quite distinct. Furthermore, the phylogram indicates a significant break between the 'mostly preblei' lineages and the 'mostly campestris' lineages, with 96% boostrap support for the node. There is significant structure in the mtDNA data set, and it *nearly* corresponds to *preblei/campestri* subspecies.

Question 2: Could you support synomyzing *Z. h.campestris* and *Z. h. preblei* without additional genetics studies (i.e. microsatellite data)? If not, what additional analysis is needed?

No, I feel more data is needed, based on the patterns of the mtDNA data presented here. DNA microsatellite data would certainly help clarify the genetic picture, although in my opinion neutral genetic markers will never provide a definitive answer to whether ecological, behavioral or physiological differences exist. But given that there is evidence for population genetic structure that largely corresponds to the traditional taxonomy, I would recommend a microsatellite study, with data analysis including Bayesian approaches to identifying cryptic population structure (eg. *Structure* analysis of Pritchard et al.). Multi-locus assignment tests should be conducted. *Z. princeps* from sympatric and allopatric populations should also be included in the analysis, to address the possibility of gene flow between these taxa.

Question 3: What is the importance of potential ecological, behavioral, or physiological differences between *Z. h. campestris* and *Z. h. preblei* in substantiating or refuting synonomy?

Such differences might exist and have been shown for closely related species and subspecies of rodents. I am not an expert on *Zapus*, and most of my information comes from the materials I was sent, and references therein. I don't think there is a clear enough understanding of the ecology and biology of these subspecies to address this question.

Question 4: What is the likelihood that the *Z. h. preblei* is substantially reproductively isolated from other groups within the *Z. hudsonius* complex, especially from *Z. h. campestris*?

If you mean could they breed together in captivity, I doubt that there is reproductive isolation. In the wild, it seems to me that the critical issue here is the range of *Z. h. preblei*, which, according to the map provided in the materials, is geographically isolated from other *Z. hudsonius* populations. There would appear to be no opportunity for gene flow from the rest of the species complex, so the population from Colorado and southeastern Wyoming (whatever its taxonomic status), *is* reproductively isolated. Furthermore, if it is lost, it will not be naturally recolonized.

Question 5: Would the loss of what is now *Z. h. preblei* represent a substantial diminution of *the Z. h. campestrix* taxon? Its Range? Biological characteristics? Evolutionary legacy? Other?

As stated in number 4 above, loss of *Z. h. preblei* would represent a loss from an important part of the species range. The rest is less clear and mostly subjective from my understanding and reading the material that was provided.